Departement für Nutztiere, Klinik für Reproduktionsmedizin
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Fate of follicular ovarian cysts in early postpartum dairy cows treated with PRID/PGF or PRID/PGF+eCG

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Vetsuisse-Fakultät Universität Zürich (2015)

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Fate of follicular ovarian cysts in early postpartum dairy cows treated with PRID/PGF or PRID/PGF+eCG

The present paper investigates the effect of progesterone administration using a progesterone-releasing intravaginal device (PRID), with or without the addition of equine chorionic gonadotropin (eCG), to cows with cystic ovarian disease (COD) in the first month postpartum. 33 dairy cows diagnosed with COD between day 20 and 30 pp were assigned to treatment groups (TG) PRID/PGF, PRID/PGF+eCG and a control group (CG). Cows of the TG received on day 0 a PRID, which was removed on day 10, at which time PGF2α was given. Cows of the PRID/PGF+eCG group received eCG on day 7. Ovarian ultrasonography and blood sampling for measurement of plasma progesterone and estradiol-17β were done over the 20-day study period. On day 0, all cows had follicular cysts, 6 of which developed into luteal cysts in the CG. The mean cyst diameter remained unchanged in the CG and decreased in the TG by day 20. All treated cows ovulated and formed a corpus luteum (CL) after PRID removal, but only 2 control cows ovulated. Of 12 ovulatory follicles in the TG dominant on day 7, 8 formed a CL with a cavity, whereas of 10 ovulatory follicles dominant on day 10, only 1 formed a CL with a cavity. The diameters of follicles, that formed a CL with a cavity, were larger on day 10. Treatment with PRID alone led to resolution of COD in all cows and therefore the additional benefit of eCG could not be critically assessed. CL with a cavity more often originate from larger and older dominant ovulatory follicles.

Key words: Cattle, cystic ovarian disease, corpus luteum, progesterone

II Zusammenfassung

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Schicksal von frühzeitig postpartum auftretenden Ovarialzysten ohne und nach Behandlung mit PRID/PGF oder PRID/PGF+eCG

Ziel der Arbeit war es, sonographisch das Schicksal frühzeitig postpartum auftretender Ovarialzysten (OZ) ohne und nach Behandlung mit einer Progesteron abgebenden Vaginalspirale (PRID), mit oder ohne zusätzlicher Applikation von equinem Choriongonadotropin (eCG), zu vergleichen. 33 Milchkühe, die 20 bis 30 Tage pp eine OZ aufwiesen, wurden in die Behandlungsgruppen (BG) PRID/PGF oder PRID/PGF+eCG sowie eine Kontrollgruppe (KG) eingeteilt. Am Tag 0 wurde den Tieren der BG eine PRID eingesetzt und am Tag 10 entfernt sowie PGF2α appliziert. In der BG PRID/PGF+eCG wurde am Tag 7 eCG appliziert. Während der 20-tägigen Studie wurden die Ovarien sonographisch untersucht sowie Progesteron und Östradiol-17β bestimmt. An Tag 0 wurden nur Follikelthekazysten festgestellt, in der KG wandelten sich davon 6 in Follikelluteinzysten um. Der Zystendurchmesser blieb in der KG unverändert, er verringerte sich in den BG bis Tag 20. Bei allen Tieren der BG kam es zur Ovulation und Anbildung eines Corpus luteum (CL), in der KG nur bei 2 Tieren. Aus bereits am Tag 7 dominanten Follikeln entstanden mehr CL mit Hohlraum als aus erstmals am Tag 10 dominanten Follikeln. Die Durchmesser der Follikel, aus denen CL mit Hohlraum entstanden, waren am Tag 10 grösser. Die Behandlung ausschließlich mit PRID führte zu einer Heilungsrate von 100 %, somit konnte der zusätzliche Nutzen von eCG nicht kritisch überprüft werden. CL mit Hohlraum entstehen häufiger aus grösseren und älteren dominanten Follikeln.

Schlüsselwörter: Rind, Ovarialzystensyndrom, Gelbkörper, Progesteron

III Manuskript

Fate of follicular ovarian cysts in early postpartum dairy cows treated with PRID/PGF or PRID/PGF+eCG

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Keywords: Cattle, cystic ovarian disease, corpus luteum, progesterone
Schlüsselwörter: Rind, Ovarialzystensyndrom, Gelbkörper, Progesteron

Summary

Objective: To investigate the effect of administration of exogenous progesterone using a progesterone-releasing intravaginal device (PRID), with or without the addition of equine chorionic gonadotropin (eCG), to cows with cystic ovarian disease (COD) in the first month postpartum ultrasonographically. **Material and method:** 33 dairy cows (n = 11/group) diagnosed with COD between day 20 and 30 postpartum were randomly assigned to treatment groups (TG) PRID/PGF, PRID/PGF+eCG and a control group (CG). The CG remained untreated during the 20-day study period, which started on the day the diagnosis was made (day 0). Cows of the TG received a PRID, which was removed on day 10, at which time PGF2α was given intravenously. Cows of the PRID/PGF+eCG group received eCG on day 7 intravenously. Ovarian ultrasonography and blood sampling for measurement of plasma progesterone (P4) and estradiol-17β (E2) were done on days 0, 7, 10, 13, and 20 in the TG and on days 0, 10, and 20 in the CG. **Results:** On day 0, all cows had follicular cysts, 6 of which developed into luteal cysts in the CG. The mean cyst diameter remained unchanged in the CG and decreased in the TG by day 20 ($p < 0.01$). All treated cows ovulated and formed at least 1 corpus luteum (CL) after PRID removal, but only 2 control cows ovulated ($p < 0.05$). Of 12 ovulatory follicles in the TG dominant on day 7, 8 formed a CL with a cavity, whereas of 10 ovulatory follicles in the same groups, dominant on day 10, only 1 formed a CL with a cavity ($p < 0.05$). The diameters of follicles, that formed a CL with a cavity, were larger on day 10 ($p < 0.01$). Mean P4 concentrations on day 0 were ≤ 1 ng/ml in all groups. P4 concentration increased during treatment and decreased below 1 ng/ml after PRID removal ($p < 0.05$). **Conclusion and clinical relevance:** Treatment with PRID alone led to resolution of COD in all cows and therefore the additional benefit of eCG could not be critically assessed. CL with a cavity more often originate from larger and older dominant ovulatory follicles.

Schicksal von frühzeitig postpartum auftretenden Ovarialzysten ohne und nach Behandlung mit PRID/PGF oder PRID/PGF+eCG

Zusammenfassung

Ziel der Arbeit war es, sonographisch das Schicksal frühzeitig postpartum auftretender Ovarialzysten (OZ) ohne und nach Behandlung mit einer Progesteron abgebenden Vaginalspirale (PRID), mit oder ohne zusätzlicher Applikation von equinem Choriongonadotropin (eCG), zu vergleichen. **Material und Methode:** 33 Milchkühe (n=11/Gruppe), die 20 bis 30 Tage postpartum eine OZ aufwiesen, wurden in die Behandlungsgruppen (BG) PRID/PGF oder PRID/PGF+eCG, sowie eine Kontrollgruppe (KG) eingeteilt. Während des 20-tägigen Zeitraums blieb die KG unbehandelt. Am Tag der Diagnose (Tag 0) wurde den Tieren der BG eine PRID eingesetzt und beim Entfernen (Tag 10) PGF2α appliziert. In der BG PRID/PGF+eCG wurde am Tag 7 eCG appliziert. Am Tag 0, 7, 10, 13 und 20 wurden die Ovarien sonografisch untersucht, sowie Progesteron (P4) und Östradiol-17β (E2) im Plasma bestimmt. Dies erfolgte bei der KG nur an Tag 0, 10 und 20. **Ergebnisse:** An Tag 0 wurden sonografisch nur Follikelthekazysten festgestellt, in der KG wandelten sich davon 6 in Follikelluteinzysten um. Der Zystendurchmesser blieb in der KG unverändert, er verringerte sich in den BG bis Tag 20 ($p < 0.01$). Bei allen Tieren der BG kam es zur Ovulation und Anbildung mindestens eines Corpus luteum (CL), in der KG nur bei 2 Tieren ($p < 0.05$). Im Vergleich zu erstmals am Tag 10 dominanten Follikeln entstanden aus bereits am Tag 7 dominanten Follikeln mehr CL mit Hohlraum ($p < 0.05$). Die Durchmesser der Follikel, aus denen CL mit Hohlraum entstanden, waren am Tag 10 größer ($p < 0.01$). Am Tag 0 waren die Mittelwerte von P4 in allen Gruppen ≤1 ng/ml. P4 stieg in den BG nach Einsetzen der PRID und fiel nach Entfernung unter 1 ng/ml ab ($p < 0.05$). **Schlussfolgerung:** Die Behandlung ausschließlich mit PRID führte zu einer Heilungsrate von 100 %, somit konnte der zusätzliche Nutzen von eCG nicht kritisch überprüft werden. CL mit Hohlraum entstehen häufiger aus größeren und älteren dominanten Follikeln.

1 Introduction

Cystic ovarian disease (COD) is one of the most common causes of reproductive failure in cattle. The cows often show anestrous, recurring estrous or ongoing signs of estrous. It increases days to first service and days to conception and is associated with increased cull rate. The incidence of COD usually varies among herds from 10 to 13 % depending on many factors (13, 20, 42) and peaks in the postpartum period (16, 44). A study of 34 lactations of 28 high-yielding Holstein Friesian cows during the first two months postpartum yielded even an incidence of COD of 26.7 % (43). Reasons for the development of COD remain nonspecific and a multifactorial etiology is suspected. Illness, genetical influences and stress are discussed as possible causes of COD (34). There have been only a few studies on the fate of untreated ovarian cysts. Newly formed ovarian cysts seem to be follicular cysts (16, 19, 29), which may persist, develop into luteal cysts, or regress following the ovulation of another follicle (19, 29, 46). The latter is considered one form of spontaneous recovery of COD (34, 36).

Because some studies have revealed a spontaneous recovery rate exceeding 60 %, which is similar to the recovery rate associated with current treatment regimens that involve GnRH, hCG, progesterone-releasing devices (PRID), or Ovsynch protocols, the effectiveness of these treatments has been called into question (22, 34, 36). In normal estrus, FSH is inducing a new follicular wave, which is sonographically visible after 2 to 4 days (1). Compared with cycling cows, cows with follicular cysts had a lower FSH-pulse frequency in plasma, and the FSH concentration in cyst fluid was lower than in preovulatory follicles (39). Furthermore, cows with COD had lower FSH basal concentrations and a significantly lower increase in plasma FSH concentration after GnRH compared with cycling cows (3). This raises the question whether the current treatment protocol of COD using a progesterone-releasing device and PGF2α could be improved by substituting FSH in form of equine choriogonadotropin (eCG), which has a high FSH activity. In anestrus cows postpartum eCG had a positive effect on the reproductive performance (15). The objective of this study was to investigate, whether cows with COD in the first month postpartum treated with exogenous progesterone using a progesterone-releasing intravaginal device (PRID), with or without the addition of eCG, have a better recovery rate than untreated cows.

Furthermore the focus is on evaluating the changes and appearance of the ovarian cysts and all other sonographically assessable follicular and luteal structures on the ovaries.

2 Materials and methods

2.1.1 Animals

Twenty-nine Swiss Braunvieh and 4 Red Holstein cows were used. Cows that had twins, dystocia, or retained fetal membranes were not used in this study. None of the 33 cows showed any clinical signs of other disease during the 20-day study period. The study was approved by the Office for Health and Consumer Protection of the canton of St. Gallen (No. SG 10/04).

2.1.2 Region and timing of study

The cows originated from 27 pasture-based dairy farms that were 650 to 1,000 meters above sea level in the north-eastern part of Switzerland. All farms were attended by the same veterinary practice. Farm size varied from 10 to 50 milking cows. The research was conducted between June 2010 and September 2011.

2.1.3 Milk yield and parity

The mean energy-corrected milk yield during the first 100 days of lactation was 2890 ± 142 kg (minimum of 1930 kg and maximum of 4720 kg). There were 3 first-lactation cows, 2 of which were in the control group and 1 in the PRID/PGF+eCG group.

2.2 Study design

Thirty-three cows with a diagnosis of COD during a reproductive examination between day 20 and 30 postpartum were randomly assigned to treatment groups PRID/PGF+eCG (n=11) and PRID/PGF (n=11) and a control group (n=11) (Fig. 1). Controls were not treated during the 20-day study period, which started on the day the diagnosis was made (day 0). Cows of the two treatment groups received a PRID (PRID alpha, 1.55 g progesterone, Biokema, Crissier, Switzerland) on day 0, which was removed on day 10, at which time PGF2α (Dinolytic, 25 mg Dinoprost, Pfizer, Zürich, Switzerland) was given intravenously (The intravenous administration of PGF2α is off-label use in Germany). Cows of the PRID/PGF+eCG group received 1000 IU eCG (Folligon. Veterinaria, Pfäffikon, Switzerland) intravenously on day 7 to induce a follicular wave, which should be present at the time of PRID removal (day 10). Cows of the treatment groups underwent ovarian ultrasonography and blood sampling for measurement of plasma concentrations of progesterone (P4) and estradiol-17β (E2) on days 0, 7, 10, 13, and 20. Ovarian ultrasonography was also conducted on day 15 for determination of ovulation. Controls were examined and sampled on days 0, 10, and 20.

2.3 Ovarian ultrasonography

A portable ultrasound machine (Scanner Falco 100 Vet, Pie Medical, Maastricht, Netherlands) with an 8-MHz linear array probe was used. A diagnosis of COD was made when a cystic structure with a mean diameter (see below) of at least 25 mm was detected on one or both ovaries in the absence of a corpus luteum (CL) on either ovary (18).

Differentiation between follicular and luteal cysts was based on characteristics of the cyst wall and echogenicity of the cyst content. Cysts with anechoic content and a wall thickness of < 3 mm were defined as follicular cysts, and those with a wall thickness of ≥ 3 mm and/or moderately echoic content as luteal cysts (10).

Cystic structures with a wall thickness of > 5 mm on day 0 were not considered luteal cysts because reliable distinction from a CL with a cavity was not possible (23). Thin-walled anechoic vesicles with a diameter of < 25 mm were considered follicles. Only dominant follicles with a diameter of > 10 mm were recorded

because the fate of smaller follicles could not be tracked using our examination protocol (40). Ovarian structures that were hypoechoic relative to the remaining ovarian tissue, had a wall thickness of > 5 mm and a mean diameter of > 20 mm were defined as functional CLs (24).

2.4 Measurement of functional ovarian structures

To determine the size of functional ovarian structures (cysts, follicles, CL), the ultrasonographic image was frozen at maximum size, and the maximum length (d_1) and the maximum width perpendicular to the length measurement (d_2) were determined using the electronic callipers. The mean of these 2 measurements was calculated for follicles and cysts, providing the mean diameters for analysis.

For CL without a cavity, the same 2 measurements were taken and the cross-sectional luteal area calculated using the formula for an ellipse ($0.5 * d_1 * 0.5 * d_2 * \pi$). For CL with a cavity. the area of the cavity was determined using the same formula and subtracted from the previously determined luteal area (23, 26). In cows with 2 CL, the cross-sectional areas were summed before analysis.

2.5 Hormone analysis

Blood was collected from a milk vein into a heparinized tube (16 IU heparin/ml blood, Sarstedt, Nümbrecht, Germany), centrifuged at 3000 rpm for 5 minutes, and the plasma stored at -20°C. Progesterone and E2 concentrations were measured at the Institute of Veterinary Physiology, Vetsuisse-Faculty University Berne, and the Research Department of Nutrition and Food Sciences, Technical University Munich, Germany, respectively. Progesterone and E2 were extracted from plasma using methyl tert-butyl ether/petroleum ether (30:70, v/v) and the hormone concentrations were determined using an enzyme immunoassay; P4 according to the method of Prakash et al. (35) and E2 according to the method of Meyer et al. (30). The sensitivities for P4 and E2 were 0.1 and 0.8 pg/ml, respectively. Intra and inter assay variation coefficients were 4.5 % and 8.5 % for P4 and 7.4 % and 8.0 % for E2.

2.6 Statistical analysis

The measurements were transferred into Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) for calculation of means and standard error of means. Absolute frequencies were used to generate frequency distributions. Relative changes in cyst diameter were based on the diameter on day 0. Differences between groups were analyzed by Kruskal-Wallis and Mann-Whitney tests and differences within a group were analyzed by the Friedman test and Wilcoxon signed rank test. In both cases, the Bonferroni correction was applied for the post-hoc tests. Differences between frequency distributions were analyzed using the Chi-square test or the Fisher‘s exact test when cell counts were below 5. A p-value < 0.05 was considered significant.

3 Results

3.1 Fate of ovarian cysts during the study period

On day 0, all 33 cows had a solitary ovarian cyst with a mean diameter of 35 ± 1.6 mm. All cysts were identified sonographically as follicular and none of the cows had a CL. The cysts remained distinct on ultrasonograms until day 20 in all cows. In the treated cows, the cysts were always identified as follicular, and in the control group, the cysts of 5 cows were identified throughout the study period as follicular based on ultrasonographic appearance and a P4 concentration of < 1 ng/ml. The remaining 6 follicular cysts in this group changed into luteal cysts by day 20 based on a P4 concentration > 1 ng/ml, moderate wall echogenicity and/or echogenicity of the cyst content.

In the control group, the mean cyst diameter decreased only minimal by 2 ± 6.5 % ($p = 0.3$) by day 20 (Fig. 2). In both treatment groups, the mean cyst diameter decreased significantly from days 0 to 20 , at which time the mean cyst diameter in group PRID/PGF+eCG was 67 ± 4.0 % and in group PRID/PGF was 70 ± 3.6 % of the original value on day 0 ($p < 0.05$). The percental changes in cyst diameter from days 0 to 10 and from days 10 to 20 did not differ between the treatment groups ($p = 0.4$) but the changes observed in the treatment groups differed significantly from the changes in the control group on day 20, on day 10 only PRID/PGF differed from the control group ($p < 0.01$) (Fig. 2).

3.2 3.2 Dominant follicles

3.2.1 3.2.1 Dominant follicles at different examination times

On day 0, 4 cows in the control group, 4 cows in the PRID/PGF+eCG group and 5 cows in the PRID/PGF group had at least 1 dominant follicle in addition to the cyst. With the exception of 2 follicles in 2 cows of each treatment group, these follicles underwent atresia by day 7. The remaining 4 follicles ovulated after day 13 and were replaced by CL with a cavity on day 20.

The fate of the dominant follicles detected on days 7 and 10, and monitored until day 20, is shown in Fig. 3. In total, 5 cows showed double ovulation (4 in the PRID/PGF+eCG group and 1 in the PRID/PGF group). Six cows of the PRID/PGF+eCG group had a dominant follicle first seen on day 7. Two of these follicles underwent atresia by day 10, and 4 persisted. These 4 follicles were replaced by 2 solid CLs and 2 CLs with a cavity by day 20. Seven cows of this group (including the two cows with a dominant follicle on day 7, which underwent atresia) had dominant follicles first seen on day 10, which ovulated and all formed solid CLs by day 20. In group PRID/PGF, 4 cows had a solid CL and 7 cows a CL with a cavity within the 20-day period.

Cows of groups PRID/PGF+eCG and PRID/PGF developed 7 and 3 new dominant follicles, respectively, from day 7 to 10, but the difference between the groups was not significant ($p = 0.08$).

Two control cows had a dominant follicle first seen on day 10. These follicles were replaced by CL (1 solid, 1 with a cavity) by day 20. The number of cows with dominant follicles on day 10 differed significantly between each treatment group and the control group ($p < 0.05$).

In the treatment groups, the day of first detection of the ovulatory dominant follicle (day 7 vs day 10) had a significant effect on the ratio of solid CL to CL with a cavity. Ovulatory dominant follicles first seen on day 7 yielded more CL with a cavity (8 of 12) than follicles first seen on day 10 (1 of 10; $p < 0.05$).

The number of cows with dominant follicles on day 20 did not differ among the 3 groups; 8, 7, and 5 cows of the PRID/PGF+eCG. PRID/PGF and control group had at least 1 dominant follicle ($p = 0.4$).

3.2.2 Size of ovulatory dominant follicle on day 10

The mean diameter of the ovulatory dominant follicle could only be determined on day 10 in all cows because 9 cows of the treatment groups had already ovulated by day 13 at the time of the examination. The mean follicular diameters in the PRID/PGF+eCG and PRID/PGF groups were 14 ± 0.8 and 18 ± 1.1 mm, respectively ($p < 0.01$). The 2 ovulatory follicles in the control group measured 15 and 16 mm. Ovarian ultrasonography on day 15 confirmed ovulation in all treated cows.

3.2.3 Type of corpus luteum and size of ovulatory follicle on day 10

The mean diameter of ovulatory follicles on day 10 that gave rise to a CL with a cavity and a solid CL was 19 ± 1.3 and 14 ± 0.7 mm, respectively ($p < 0.01$).

3.3 Corpora lutea

Corpora lutea were seen only on day 20. All treated cows had at least 1 CL, in the control group only 2 cows had one CL (Table 1; $p < 0.001$). Because of 4 double ovulations in group PRID/PGF+eCG and 1 in group PRID/PGF, the total number of CLs in these two groups was 15 and 12, respectively. All CLs from double ovulations were solid. All but 1 cow with at least 1 CL had P4 concentrations > 1 ng/ml; in 1 cow of the PRID/PGF+eCG group, a CL with a 5 mm wall and 3.3 cm^2 cavity was accompanied by a P4 concentration of 0.7 ng/ml.

The mean luteal area was larger in the PRID/PGF+eCG group than in the PRID/PGF group (7.0 ± 0.90 cm^2 vs 4.6 ± 0.51 cm^2; $p < 0.05$). The luteal area of the 2 CLs in the control groups measured 6.5 and 4.9 cm^2.

More CL with a cavity than solid CL were present in the PRID/PGF group in comparison to the PRID/PGF+eCG group (Table 1; $p < 0.05$). The cross-sectional area and maximum length of the luteal cavity did not differ between the 2 treatment groups (PRID/PGF+eCG, 2.8 ± 0.58 cm^2 and 30 ± 5.0 mm; PRID/PGF. 3.6 ± 0.85 cm^2 and 24 ± 3.2 mm, $p = 0.6$ respectively $p = 0.5$). Two cows in the PRID/PGF group had noticeable large luteal cavities measuring 6.9 cm^2 and 5.8 cm^2. Although these structures had a wall that measured only 3 mm in some parts, they were identified as CL because ovulation was detected in both cases on day 15. The P4 concentrations of these cows were 6.5 and 2.3 ng/ml.

3.4 3.4 Progesterone and Estradiol-17-β concentrations

3.4.1 Progesterone

The P4 concentrations on day 0 measured 0.3 ± 0.13 ng/ml in the controls, 0.7 ± 0.13 ng/ml in the PRID/PGF+eCG group. and 1.0 ± 0.32 ng/ml in the PRID/PGF group. Five cows (2 each in the treatment groups and 1 in the control group) had P4 concentrations > 1 ng/ml with a mean of 1.7 ± 0.23 ng/ml.

P4 changed in all groups significantly during the study period ($p < 0.05$). There was a significant increase in P4 concentration between day 0 and 7 in both treatment groups from 0.7 ± 0.13 ng/ml to 2.1 ± 0.68 ng/ml (PRID/PGF+eCG) and from 1.0 ± 0.32 ng/ml to 2.1 ± 0.45 ng/ml (PRID/PGF) ($p < 0.05$). There was no change between day 7 and 10, a decrease between day 10 and 13 (PRID/PGF+eCG, from 2.1 ± 0.84 ng/ml to 0.5 ± 0.07 ng/ml, $p < 0.05$; PRID/PGF, from 1.5 ± 0.17 ng/ml to 0.3 ± 0.06 ng/ml. $p < 0.05$), and an increase by day 20 to 14.0 ± 6.27 ng/ml (PRID/PGF+eCG, $p < .0.05$) and 5.4 ± 0.72 ng/ml (PRID/PGF, $p < 0.05$). In the control group only the increase of P4 from day 0 to day 20 (0,7 ± 0,37 ng/ml to 2,9 ± 0,87 ng/ml) was significant, as it was in both treatment groups (all $p < 0.05$).

3.4.2 Estradiol-17β

E2 only changed significantly in the PRID/PGF+eCG group during the study period ($p < 0.05$). E2 increased significantly between day 7 and 10 from 10.8 ± 1.12 pg/ml to 17.4 ± 2.37 pg/ml ($p < 0.01$).

4 Discussion

At the time of diagnosis, all cysts were classified as follicular based on ultrasonographic criteria. Classification is aided by P4 concentration (5); concentrations ≥ 1 ng/ml are generally associated with luteal cysts. Five of 33 cows (15 %) had P4 concentrations ≥ 1 ng/ml but showed no sonographical signs of lutenization in the ultrasonographic examination.

Hasler et al. (19) and Leidl et al. (28) found that all or more than 90 % of ovarian cysts were follicular. In contrast, luteal cysts comprised 40 % of ovarian cysts in a study by Probo et al. (37), and more than 60 % in a report by Farin et al. (12). This discrepancy could be related to the time of examination because follicular cysts may become luteinized over time (6, 19, 29). It is conceivable that cows with follicular cysts diagnosed by ultrasonography and which had increased P4 plasma concentrations, the cysts were in the early stages of luteinization. Thus, reliable differentiation of follicular and luteal cysts is not always possible, even with the combination of ultrasonographic criteria and the P4 concentration. This was exemplified in 6 control cows, in which the follicular cysts underwent luteinization.

All treated cows and 2 control cows ovulated and formed a CL between day 10 and 20. By definition, this marks the resolution of COD and the end of ovarian dysfunction (2, 7). Because of the low number of cases per herd and group and the high regression rate of COD in both treatment groups, no final conclusion can be drawn with regard to equality or superiority of one of the two treatments. Factors which might have affected the treatment response, for example milk yield (9), negative energy balance (21) or differences in the body condition (8), could not be assessed in the present study.

In all but one of the cows, the initiation of a new cycle was associated with a massive reduction in cyst diameter by a mean of 30 %. A reduction in cyst size associated with the start of a new cycle was observed by others (2, 7, 38), but information about the magnitude was not given. Only Calder et al. (4) induced COD in cows experimentally using a 7-day course of E2 and P4. Subsequent treatment of the cyst with a PRID/PGF led to a reduction in cyst diameter of 15 %, which was similar to the mean reduction of 13 % seen in the treated cows of this study. By contrast, the transformation of follicular cysts into luteal cysts in 6 controls of this study was on average not associated with a reduction in cyst diameter. Luteal cysts are described to have a lifespan comparable to a cyclic CL and regress spontaneously, and thus appear to affect cyclic activity only transiently, luteinization of a follicular cyst could therefore be considered a form of follicular cyst resolution (17, 47). But unlike the follicular cysts of the treated cows in this study, cyst resolution via luteinization may not necessarily be associated with a reduction in diameter, at least in the early stages.

At the time of diagnosis of COD, 61% of cows did not have a dominant follicle, suggesting that the cysts suppressed the selection of a dominant follicle. According to Kaneko et al. (25), ovarian cysts have a longer period of dominance than emerging dominant follicles. On days 10 and 20, the cysts of 9 treated cows and 6 control cows, respectively, were not accompanied by a dominant follicle, whereas on day 7 and 10, 13 and 22 treated cows, respectively, had at least 1 dominant follicle. In cows with experimentally-induced ovarian cysts that were treated with a PRID for 9 days, a new follicular wave emerged within 3 to 8 days after the start of treatment (4).

Cows treated with eCG on day 7 had more emerging dominant follicles between day 7 and 10 that ovulated by day 13 than cows treated with PRID only. Mihm et al. (31) showed that only dominant follicles with a period of dominance of < 4 days are associated with an optimal conception rate. Further studies are needed to evaluate, if eCG in addition to PRID and PGF2α improves first service conception rate in cows with OCD.

An interesting observation in this context were the 12 dominant follicles, first seen on day 7 or even on day 0, that ovulated after the prolonged time of dominance by day 15. Subluteal P4 concentrations of about 2 ng/ml, typical of cows treated with a PRID, cause persistence, size increase, and aging of the dominant follicle (41). In the present study, 66 % of these ‚persistent' but ovulatory follicles formed a CL with a cavity.

These follicles were significantly larger before ovulation on day 10 than follicles that resulted in solid CLs, which was in agreement with observations in 22 cows with spontaneous estrus that larger ovulatory follicles tend to form CLs with a cavity more frequently than smaller ovulatory follicles (33). Cycle length, P4 concentration, and fertility in the subsequent estrus do not differ between solid CLs and CLs with a cavity (23, 27). Only few studies have investigated characteristics of the estrous cycles that precede the formation of a CL with a cavity. Mihm et al. (31) extended the period of dominance of the ovulatory follicle from 4 to 10 days using a norgestomet ear implant and observed an increase in size of the ovulatory follicle and a four-fold increase in the luteal cavity area compared with controls. Fralix et. al. (14) treated multiparous Angus cows orally with melengestrol acetate

for 14 days, which led to the formation of CLs with cavities that were twice the size of the luteal cavities in the following normal cycle.

The cavities of the CLs showed a large range in size (from 0.6 to 6.9 cm^2) with a mean of 3.1 cm^2 compared to the mean of this area in 69 anestrous dairy cows with only 1 cm^2 (45). The length of the luteal cavity of cows with a normal cycle was usually less than 15 mm and only rarely exceeded 20 mm (23), whereas in 6 of the 9 CLs with a cavity in the treatment groups of the present study, the cavity length was greater than 25 mm. Studies are needed to clarify whether the presence of a CL with a cavity and/or the area of the luteal cavity allow a conclusion with respect to size and age of the preceding ovulatory follicle.

All treated cows responded to treatment because all had at least 1 CL on day 20. Excluding the cow with a CL accompanied by a P4 concentration of < 1 ng/ml yields still a success rate of 91 %, which is comparable to success rates of 82 % (48) and 90 % (36) in dairy cows with follicular cysts diagnosed after 40 days postpartum and treated with a PRID. The cows of the present study were treated during the first month postpartum, during which time spontaneous recovery is more common (14), this could yield to a higher treatment success.

Spontaneous recovery occurred in only 2 cows of the control group, in contrast to a spontaneous recovery rate of 81 % in 339 dairy cows with follicular cysts (36). In that study, the diagnostic criterion was a cystic structure with at least 25 mm in length measured ultrasonographically on a single occasion. Our criterion was a mean cyst diameter of > 25 mm, and it is therefore possible that some follicular cysts were mistaken for oversized aged ovulatory follicles in the study by (36). Furthermore, 68 % of all cysts in that study were no longer visible at re-examination 2 to 4 weeks after the diagnosis, whereas all cysts remained visible ultrasonographically until day 20 in the present study.

Double ovulations occurred in 4 cows of the PRID/PGF+eCG and in 1 cow of the PRID/PGF group. Increased numbers of multiple ovulations in cows treated with eCG as part of a synchronization program were also observed by Duffy et al. (11). Double ovulations bear the risk of twin pregnancies and associated problems including abortion, retained placenta, infertility, and increased calving interval (32).

Treatment of cows with COD in the first month postpartum using a PRID and PGF2α was successful in all cases and therefore the additional benefit of eCG to the treatment regimen could not be critically assessed. Equine CG stimulates the development of new dominant follicles and results in ovulation of smaller follicles. There was a striking increase in the occurrence of CLs with a cavity when no eCG was used and after the ovulation of larger follicles. Studies are needed to investigate whether the occurrence of a CL with a cavity and/or the size of the area of the cavity allow conclusions regarding the size and age of the original ovulatory follicle.

5 Conclusion for practice

Treatment of cows with COD in the first month postpartum using a PRID and PGF2α was successful in all cases. Additional treatment with eCG leads to more new dominant follicles and ovulation of smaller follicles. Corpora lutea with large cavities were seen after ovulating of larger follicles, as well as when cows were treated only with PRID and PGF2α, without eCG.

Competing interests

The authors declare that they have no competing interests.

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Figures and tables legend

Fig. 1. Schedule of ovarian ultrasonographic examination and blood sampling (🡻) in 2 treatment groups and control group.

✯ Ovarian ultrasonography for examination of ovulation

Fig. 2. Relative changes in cyst diameter in cows of 2 treatment groups and a control group during the study period.

a,b: values within days with different indices are different ($p < 0.05$)

*: values within groups differ from preceding measurement ($p < 0.05$)

Fig. 3. Fate of dominant follicles from days 7 to 20 in 2 treatment groups and control group of cows with COD diagnosed between day 20 and 30 postpartum.

◯ Dominant follicle ⃠ follicular atresia ● solid CL ◉ CL with a cavity

Fig. 4. Progesterone concentration in cows diagnosed with COD between day 20 and 30 postpartum treated with PRID/PGF+eCG or PRID/PGF and in untreated controls.

* means within groups differ from preceding means ($p < 0.05$).

Fig. 5. Estradiol-17β concentration in cows diagnosed with COD between day 20 and 30 postpartum treated with PRID/PGF+eCG or PRID/PGF and in untreated controls.

* means within groups differ from preceding means ($p < 0.05$).

Table 1: Number, size, and type of corpora lutea in cows of 2 treatment groups and 1 control group.

a,b: numbers within columns with different indices are different ($p < 0.05$).

Tables

Total number of cows in study (n = 33)	Number of cows with CL	Number of CL	Luteal area (cm^2)	Corpora lutea with cavity	
				Number of CL	Cross-sectional area of cavity (cm^2)
PRID/PGF+eCG (n=11)	11 [a]	15 [a]	7.0 ± 0.90 [a]	2 [a]	2.8 ± 0.58 [a]
PRID/PGF (n=11)	11 [a]	12 [a]	4.6 ± 0.51 [b]	7 [b]	3.6 ± 0.85 [a]
Controls (n=11)	2 [b]	2 [b]	5.7 ± 0.78 [a,b]	1 [a,b]	0.6 [a]

Abbildungs- und Tabellenlegende

Abb. 1: Versuchsanordnung der beiden Behandlungsgruppen PRID/PGF+eCG und PRID/PGF sowie der Kontrollgruppe.

↓ Zeitpunkt der sonografischen Untersuchung der Ovarien und der Blutentnahmen

✡ sonografische Untersuchung der Ovarien zur Ovulationskontrolle

Abb 2: Prozentuale Änderung der Zystendurchmesser in den 2 Behandlungsgruppen PRID/PGF+eCG und PRID/PGF sowie der Kontrollgruppe über den 20-tägigen Untersuchungszeitraum.

a.b: Werte mit unterschiedlichen Indices am gleichen Versuchstag unterscheiden sich signifikant.

*: In der gleichen Gruppe ist die Änderung verglichen mit dem vorangehenden Wert signifikant.

Abb. 3: Schicksal der dominanten Follikel von Tag 7 bis Tag 20 in den 2 Behandlungsgruppen PRID/PGF+eCG, PRID/PGF sowie der Kontrollgruppe über den 20-tägigen Untersuchungszeitraum.

○ dominanter Follikel ⊘ Follikelatresie ● kompaktes CL ◉ CL mit Hohlraum

Abb. 4: Verlauf der Progesteronkonzentrationen der 2 Behandlungsgruppen PRID/PGF+eCG und PRID/PGF sowie der Kontrollgruppe.

* In der gleichen Gruppe ist die Änderung verglichen mit dem vorangehenden Wert signifikant ($p < 0.05$).

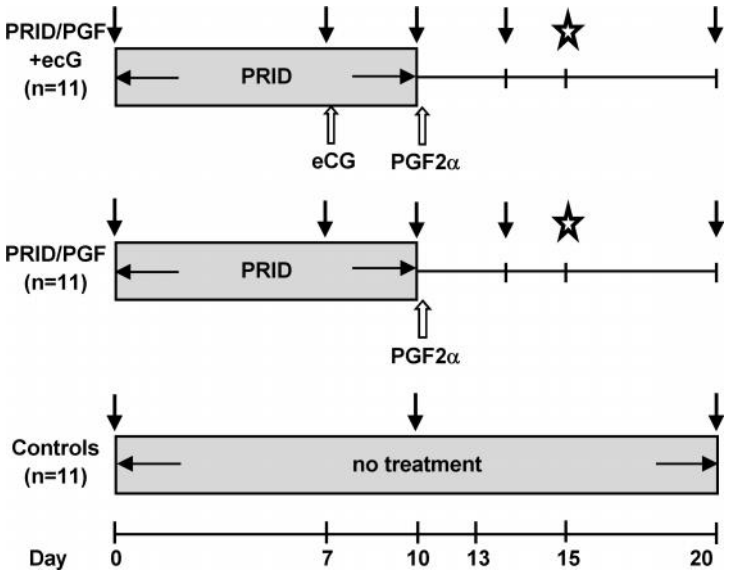
Abb. 5: Verlauf der Östradiol-17β- Konzentrationen den Behandlungsgruppen PRID/PGF+eCG, PRID/PGF sowie der Kontrollgruppe.

* In der gleichen Gruppe ist die Änderung verglichen mit dem vorangehenden Wert signifikant ($p < 0.05$).

Tab. 1: Anzahl, Größe und Form der Corpora lutea in den Behandlungsgruppen PRID/PGF +eCG, PRID/PGF sowie der Kontrollgruppe am Ende des 20tägigen Untersuchungszeitraums.

a,b: Werte mit unterschiedlichen Indices in der gleichen Spalte unterscheiden sich signifikant ($p < 0.05$).

Figures



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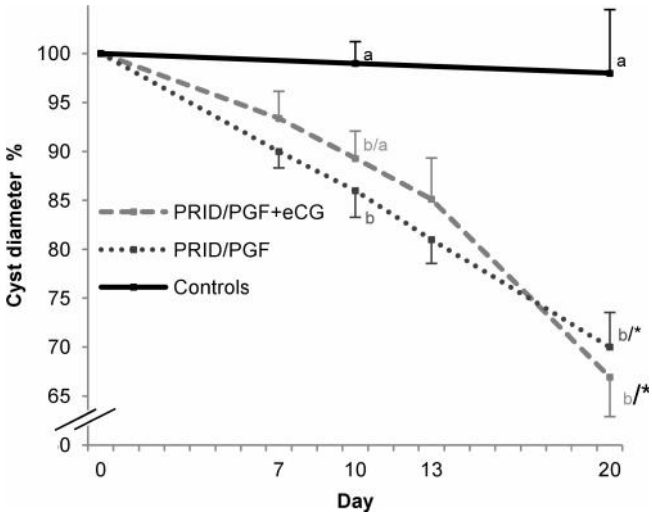

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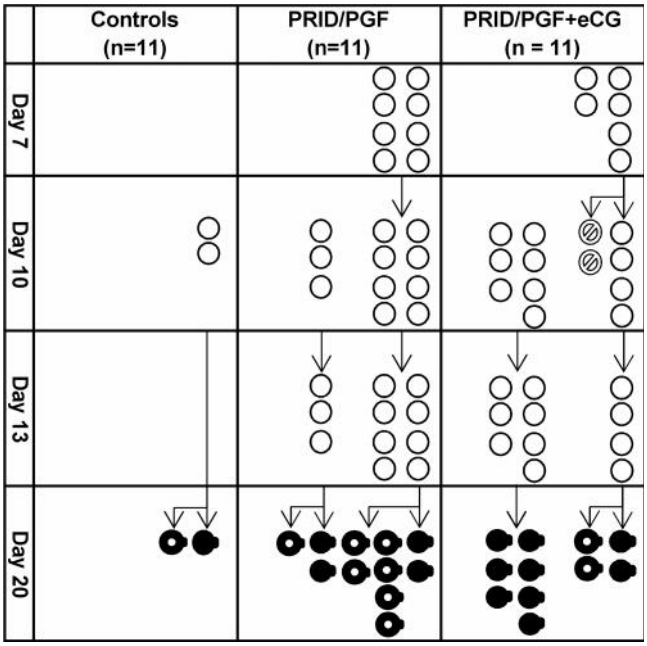
a,b: values within days with different indices are different ($p < 0.05$)

*: values within groups differ from preceding measurement ($p < 0.05$)



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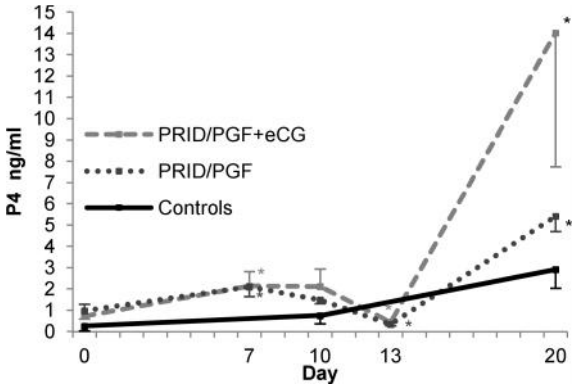

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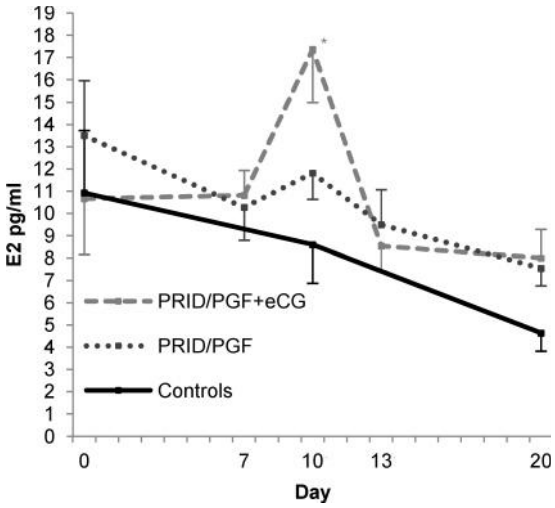

Fig. 5. Estradiol-17β concentration in cows diagnosed with COD between day 20 and 30 postpartum treated with PRID/PGF+eCG or PRID/PGF and in untreated controls.

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